Isolation of Dolastatin 10 from the Marine Cyanobacterium *Symploca* Species VP642 and Total Stereochemistry and Biological Evaluation of Its Analogue Symplostatin 1

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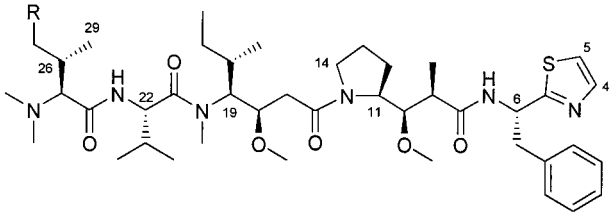
The potent antitumor agent dolastatin 10 (**1**) was originally isolated from the sea hare *Dolabella auricularia*, and we now report its isolation from the marine cyanobacterium *Symploca* sp. VP642 from Palau. The chemically related analogue symplostatin 1 (**2**) has been reisolated from Guamanian and Hawaiian varieties of *S. hydnoides* and its total stereochemistry completed by determining the *N*,*N*-dimethylisoleucine unit to be L. Symplostatin 1 (**2**), like dolastatin 10 (**1**), is a potent microtubule inhibitor. The antitumor activity of **2** was assessed in vivo against several murine tumors. Symplostatin 1 (**2**) was effective against a drug-insensitive mammary tumor and a drug-insensitive colon tumor; however, it was only slightly effective against two MDR tumors.

The dolastatins are cytotoxic compounds that have been isolated in low yields (10^{-6} to 10^{-7}%) from the sea hare *Dolabella auricularia* by the research groups of Pettit[1] and Yamada.[2] Dolastatin 10 (**1**) is currently in clinical trials and therefore of considerable interest.[3] The cyanobacterial, dietary origin of several dolastatins[4] and other compounds[5] obtained from *D. auricularia* has been recently demonstrated. We have already reported the isolation of the dolastatin 10 analogue symplostatin 1 (**2**)[4b] and have now, unsurprisingly, encountered dolastatin 10 (**1**) itself in one of our Palauan cyanobacterial collections (~10^{-2}% dry wt). In this paper, we also report the results of the biological evaluation of symplostatin 1 (**2**) in comparison with dolastatin 10 (**1**) and the results of the in vivo testing of symplostatin 1 (**2**).



1 R = H
2 R = CH_3

Results and Discussion

The lipophilic extract of *Symploca* sp. VP642 (VP642L) collected at Ulong Channel, Palau, in April 1999, showed murine and human solid tumor selective cytotoxicity in the Corbett assay[6] (Table 1) and also appeared to be extremely potent (dilutions of 1/500 produced inhibition zones >500 units). Because of this promising profile, the extract was selected for in vivo testing. Preliminary trials were conducted (by the iv route) to determine dose-limiting toxicity. Lethal toxicity occurred with a dosing schedule of 6 mg/kg/inj on day 3 and 4 for a total dose of 12 mg/kg. The mice precipitously lost weight, their appearance degenerated, and all were dead by day 9 (LD_{100}). Necropsy results were consistent, revealing an empty, inflamed stomach, and diarrhea, indicating gastrointestinal (GI) epithelial damage. At sublethal doses, VP642L showed high activity against early stage mammary adenocarcinoma 16/C and modest activity against early stage pancreatic ductal adenocarcinoma P03 (Table 2), justifying further evaluation.

The aqueous extract VP642A exhibited lower potency, but it showed the same profile as VP642L in the Corbett assay (Table 1). Thus we had reason to believe that the active component in both extracts was the same, and so the cytotoxin from VP642A was isolated. Solvent partition between *n*-BuOH and H_2O concentrated the cytotoxicity in the organic phase. The ^{1}H NMR spectrum of the CH_2Cl_2-soluble material of the organic portion revealed the presence of almost exclusively either dolastatin 10 (**1**) or symplostatin 1 (**2**), or another closely related analogue. Reversed-phase HPLC using the system from Pettit et al.[7] then yielded the pure compound, which was identified to be dolastatin 10 (**1**) by spectral comparison (HRMS, ^{1}H and ^{13}C NMR, $[\alpha]_D$, UV) with the known compound.

In April 2000 we attempted to re-collect the dolastatin 10-producing organism at the same collection site. Although the presumed producer was less abundant, the re-collection yielded a highly cytotoxic extract that appeared to contain about 30 pg of dolastatin 10 (**1**); however, loss of activity of the small sample during chromatographic fractionation precluded a rigorous identification of the cytotoxin (e.g., by LCMS). The sequencing of the 16S ribosomal RNA gene indicated that the re-collection was an assemblage, even though homogeneous in appearance, of three different cyanobacteria, designated VP642a−c (GenBank accession nos. AY032932−AY032934). Consequently we are uncertain about the actual dolastatin 10 source(s). VP642b and VP642c displayed 99.3% similarity to each other; however, VP642a differed to a greater extent by showing 95.5% similarity to VP642b and 95.0% similarity to VP642c. The gathering of three distinct cyanobacteria

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Table 1. Evaluation of VP642 Extracts in the Differential Cytotoxicity (Corbett) Assay[a]

		leukemia	mouse solid tumors		human solid tumors			normal cells
extract	dilution	L1210	P03	C38	H15	H116	H125	fibroblast
VP642L	1/500	600	920	870	930	950	0−220	0−900
VP642A	1/25	610	800−900		900			0−920

[a] Zone units of inhibition (200 units = 6.5 mm). Extracts are solid tumor selective if zone differentials between solid tumors and leukemia/normal cells are >250 units.

Table 2. In Vivo Activity of VP642L Administered Intravenously against Early Stage Subcutaneous Implanted Solid Tumors

sc tumor	schedule	total dosage[a] (mg/kg)	T/C[b](%)	gross log cell kill[c]	activity rating[d]
murine mammary 16/C	d 1−3	6	3	2.3	highly active +++
murine pancreatic 03	d 3−5, 7−9	12	33	1.9	modestly active ++

[a] Near to maximum tolerated dosage in the strain of mouse used in the trial. [b] T/C values that are less than 42% are considered to be active by NCI standards; T/C values that are less than 10% are considered to have excellent activity and potential clinical activity by NCI standards. [c] Defined as $T - C/(3.32)(T_d)$ where T is the median time in days for the tumors of the treated group of mice to reach 750−1000 mg, C is the median time in days for the tumors of the control group to reach 750−1000 mg, and T_d is the tumor volume doubling time. [d] Based on log cell kill.[14] An activity rating of +++ to ++++, which is indicative of clinical activity, is needed to effect partial or complete regression of 100−300 mg sized masses of most transplanted solid tumors of mice.

during the re-collection could have resulted from our inability to discern subtle differences in these organisms because of technical difficulties experienced during the dives (e.g., strong currents). The 16S rRNA gene analysis described herein illustrates some of the problems or hindrances that one encounters in field collections of cyanobacteria.

Symplostatin 1 (**2**)[4b,8] had been isolated earlier from Guamanian *S. hydnoides* VP377 and was reisolated from a re-collection made in November 1998 for the work described herein.[9] Its structure differs from dolastatin 10 (**1**) by only one additional CH_2 unit in the *N*-terminal residue (L-Me_2Val substituted by L-Me_2Ile). The absolute configuration of the remaining stereocenter (C-26) in **2** was established to be 26*S* by comparing the HPLC retention time of the *N*-terminal amino acid from hydrolysis with those of synthetic samples of L-Me_2Ile, D-Me_2Ile, L-*allo*-Me_2Ile, and D-*allo*-Me_2Ile. The sequencing of the 16S ribosomal RNA gene of *S. hydnoides* VP377 led us to conclude that only one cyanobacterium had been collected (GenBank accession no. AF306497). Its sequence closely matched the ones for VP642b (98.9% similarity) and VP642c (98.7% similarity), so that the latter two organisms from Palau are probably varieties of the species *S. hydnoides*. Comparison of VP377 with Palauan VP642a demonstrated 95.2% similarity, suggesting a species difference for the latter organism. It should be noted that *S. hydnoides* VP377 populates the reef flat of Pago Bay, a consistent habitat over a relatively large area, easily accessible by foot at low tide so that the collection does not require scuba diving. These conditions are therefore quite different from the ones under which *Symploca* sp. VP642(a−c) were collected at Ulong Channel, Palau.

The cytotoxicity IC_{50} values of 0.052 and 0.076 nM against KB and LoVo cells, respectively, for our **1** corresponded closely with the data for synthetic dolastatin 10 (**1**) (0.041 and 0.066 nM, respectively). Evaluation of the in vitro cytotoxicity of symplostatin 1 (**2**) against KB cells (IC_{50} 0.15−0.20 nM) and LoVo cells (IC_{50} 0.34−0.50 nM) revealed that **2** is a very potent cytotoxin, but not as potent as dolastatin 10 (**1**), whereas synthetic analogues lacking the *N*,*N*-dimethylamino acid residue are reported to be markedly less cytotoxic.[10] The importance of this residue for activity had been suggested in these previous studies, but no correlation between size of the side chain and antitumor activity was detected.[10b]

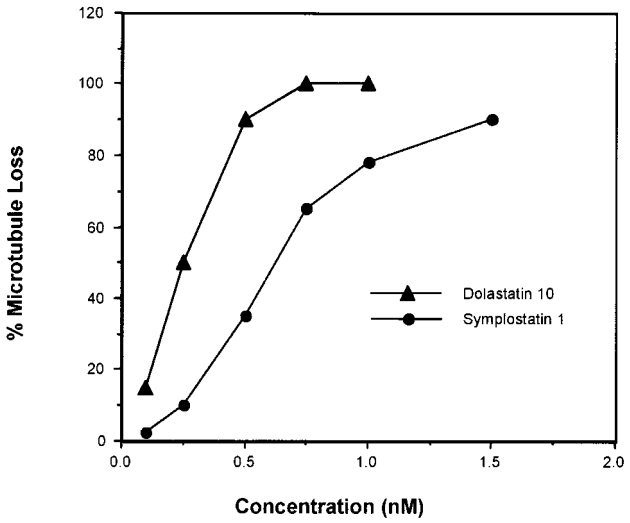

Figure 1. Effect of dolastatin 10 (**1**) and symplostatin 1 (**2**) on the loss of cellular microtubules. The effects of these two drugs were examined by indirect immunofluorescence in A-10 cells following an 18 h incubation.

The effects of dolastatin 10 (**1**) and symplostatin 1 (**2**) were examined in A-10 cells by indirect immunofluorescence. Both drugs are potent microtubule depolymerizers. Dolastatin 10 (**1**) at a concentration of 0.75 nM caused the total loss of cellular microtubules, while a 2.5 nM concentration of symplostatin 1 (**2**) was required for the same effect. Both agents caused the formation of abnormal multipolar mitotic spindles and the breakdown of the nucleus into micronuclei. The effects of the two drugs on cellular microtubules were indistinguishable, yet as predicted from the IC_{50}'s for cytotoxicity, dolastatin 10 (**1**) was more potent (Figure 1), with an approximate EC_{50} for loss of cellular microtubules of 0.25 nM, while symplostatin 1 (**2**) had an approximate EC_{50} of 0.6 nM. To put the potency of these two agents into perspective, it should be noted that, in the A-10 cell line, a 150 nM concentration of vinblastine is required to cause total microtubule loss.

Symplostatin 1 (**2**) proved to be highly potent in vivo, but also highly toxic. For example, single doses of 1.0 or 1.4 mg/kg injected intravenously into mice on day 1 caused lethality (LD_{20} and LD_{60}, respectively). Symptoms were similar to those seen after treatment with the dolastatin 10-containing VP642L extract, and necropsy results indi-

Table 3. In Vivo Activity of Symplostatin 1 (**2**) Administered Intravenously against Early Stage Subcutaneous Implanted Solid Tumors

sc tumor	schedule	total dosage[a] (mg/kg)	T/C[b] (%)	gross log cell kill[c]	activity rating[d]
murine colon 38	d 3	3	0	1.81	active ++
murine mammary 16/C	d 1, 4−6	1.25	0	2.09	highly active +++
murine mammary 17/Adr	d 1, 4, 7, 8	2.8	32	0.41	marginally active ±
murine mammary 16/C/Adr	d 1, 3, 5−9	3.15	36	0.42	marginally active ±

[a−d] See footnotes in Table 2.

cated GI toxicity and liver toxicity. Compound **2** caused severe local tissue damage in the region of the injection site. At sublethal doses symplostatin 1 (**2**) showed significant activity against two drug-insensitive murine solid tumors: early stage colon adenocarcinoma #38 and early stage mammary adenocarcinoma 16/C (Table 3). Two adriamycin-resistant murine early stage solid tumors, mammary adenocarcinoma 17/Adr and mammary adenocarcinoma 16/C/Adr, were selected to evaluate the efficacy of symplostatin 1 (**2**) against MDR tumors. Only marginal antitumor activity was observed (Table 3).

The biological evaluation of symplostatin 1 (**2**) revealed that it is highly active against certain tumors and comparable in its activity with dolastatin 10 (**1**) and isodolastatin H,[11] a dolastatin 10 analogue isolated by the Yamada group. Unfortunately these compounds display harsh toxicity and poor host recovery behavior, and hence there is the need for more efficacious analogues. The isolation of dolastatin 10 (**1**) from a marine cyanobacterium suggests that one or more cyanobacteria are the ultimate producers of **1** found in the sea hare *D. auricularia*.

The dietary origin of many compounds isolated from sea hares is now well documented, and it is our hope that this fact will prompt the medical community to obtain metabolites of interest for extensive drug evaluation not through excessive collections of the animals that accumulate these cytotoxins through diet, but rather by collection of the cyanobacteria or algae which they feed upon. This will not only reduce the ecological impact but also frequently lead to higher yields (factor ca. 10^4).

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C) and compared with the ones of the known compounds. HRFABMS were measured in the positive mode. Standards of *N*,*N*-dimethylisoleucine for the chiral HPLC analysis have been synthesized by an established method.[12]

Biological Material. Cyanobacterium *Symploca* sp. VP642 was collected at Ulong Channel, Palau, in April 1999, by scuba diving at ca. 10 m. The re-collection of VP642(a−c) at the same site was carried out in April 2000. The original isolation of symplostatin 1 (**2**) from cyanobacterium *S. hydnoides* VP377 has been described.[4b] The symplostatin 1-producing *S. hydnoides* was re-collected at the reef flat of Pago Bay, Guam, in November 1998. Specimens of the organisms were preserved in formalin and deposited in the University of Guam Marine Laboratory.

Isolation of Dolastatin 10 (1). The freeze-dried organism VP642 (∼5 g) was extracted with CH_2Cl_2 to yield the lipophilic extract VP642L (23.7 mg) and subsequently with EtOH−H_2O (3:7) to give the aqueous extract VP642A (∼1 g). VP642A was partitioned between *n*-BuOH and H_2O. The CH_2Cl_2-soluble portion (2.3 mg) of the solvent-evaporated organic phase was subjected to reversed-phase HPLC (Econosil C_8, 10 μm, 10 × 250 mm, 3.0 mL/min; UV detection at 220 nm) using the isocratic system employed by Pettit et al.[7] for dolastatin 10 (**1**) analysis [MeCN−*i*-PrOH−5 mM $HexSO_3Na/H_2O$ (65:15:20)]. Compound **1** eluted at t_R 15.5 min. To remove the mobile-phase additive, the solvent mixture was evaporated and the residue partitioned between H_2O and CH_2Cl_2; the latter phase yielded 0.9 mg of dolastatin 10 (**1**).

The freeze-dried cyanobacterial assemblage VP642a−c (∼1 g) was extracted as described above to yield a lipophilic extract (8.0 mg); however, cytotoxicity was lost during the normal-phase (Si gel) chromatography step using the procedure described below for the isolation of symplostatin 1 (**2**).

Dolastatin 10 (1): colorless gum; $[\alpha]^{25}_D$ −55° (*c* 0.20, MeOH) {lit.[3a] $[\alpha]^{29}_D$ −68° (*c* 0.01, MeOH)}; UV (MeOH) λ_{max} (log ϵ) 203 (4.39), 245 (3.63); ^{1}H NMR, ^{13}C NMR data/spectra, see refs 3a and 4b; HRFABMS *m*/*z* $[M + H]^+$ 785.5029 (calcd for $C_{42}H_{69}N_6O_6S$, 785.4999).

Isolation of Symplostatin 1 (2). VP377, re-collected in November 1998, was consecutively extracted with EtOAc and EtOH to yield VP377L (1.579 g) and VP377A (7.277 g), respectively. VP377L was partitioned between hexane and 80% aqueous MeOH. The solvent of the latter phase was evaporated and the residue partitioned between *n*-BuOH and H_2O. The *n*-BuOH portion was concentrated and subjected to Si gel chromatography eluting first with CH_2Cl_2 and followed by CH_2Cl_2 solutions containing progressively increasing amounts of MeOH. The fraction eluting with 8% MeOH in CH_2Cl_2 was applied to a C_{18} Sep Pak and elution initiated with 10% aqueous MeCN. The 60−100% MeCN fractions were combined and concentrated to dryness. The CH_2Cl_2-soluble portion was applied to a Phenyl Sep Pak and washed with CH_2Cl_2, followed by MeOH. The MeOH fraction was chromatographed on a SiO_2 Sep Pak, eluting with EtOAc, followed by EtOAc−MeOH mixtures containing progressively increasing amounts of MeOH. The fraction eluting with 1% MeOH in EtOAc was subjected to reversed-phase HPLC and subsequent solvent partition as described above for dolastatin 10 (**1**). Symplostatin 1 (**2**) (2.2 mg) eluted at t_R 15.5 min.

VP377A was partitioned between *n*-BuOH and H_2O. The concentrated organic phase was applied to Si gel chromatography employing the step gradient used above for VP377L. Without further purification, the fraction eluting with 8% MeOH in CH_2Cl_2 was subjected to reversed-phase HPLC and solvent partition as described above, yielding pure symplostatin 1 (**2**) (2.8 mg).[9,13]

Acid Hydrolysis and Amino Acid Analysis of 2. A sample of compound **2** (0.3 mg) was treated with 6 N HCl at 110 °C for 18 h. The hydrolyzate was concentrated to dryness and analyzed by chiral HPLC [column, Chirex phase 3126 (D) (4.6 × 250 mm), Phenomenex; solvent, 2 mM $CuSO_4$; flow rate, 0.8 mL/min; detection at 254 nm]. *N*,*N*-dimethylisoleucine (Me_2Ile) eluted at t_R 33.5 min, corresponding to the retention time of the authentic standard of L-Me_2Ile [t_R (min) of other standards: D-Me_2Ile (48.0), L-*allo*-Me_2Ile (37.5), D-*allo*-Me_2Ile (57.0)]. This result was further confirmed by co-injection. Expectedly, L-valine was detected under these conditions as well, eluting at t_R 28.8 min [t_R (min) of standards: L-Val (28.8), D-Val (52.0)].

Cytotoxicity Assays and in Vivo Trials. Extracts VP642L and VP642A were tested in solid tumor selectivity assays that

were performed as described previously.[6] In vivo trials with VP642L and pure symplostatin 1 (**2**) were carried out as described previously.[14] The IC_{50} values for cytotoxicity in vitro were determined using the SRB assay.[15]

Microtubule-Depolymerizing Activity. The effects of symplostatin 1 (**2**) and dolastatin 10 (**1**) were examined in A-10 cells. The cells were plated onto glass coverslips and allowed to attach and grow for at least 24 h. The drugs were added, and after an 18 h incubation the cells were fixed and cellular microtubules examined by indirect immunofluorescence.[16]

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References and Notes

(1) (a) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Fujii, Y.; Kizu, H.; Boyd, M. R.; Boettner, F. E.; Doubek, D. L.; Schmidt, J. M.; Chapuis, J.-C.; Michel, C. *Tetrahedron* **1993**, *49*, 9151−9170. (b) Pettit, G. R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Kirby, G. W., Moore, R. E., Steglich, W., Tamm, C., Eds.; Springer-Verlag: Vienna, New York, 1997; Vol. 70, pp 1−79.

(2) Sone, H.; Kigoshi, H.; Yamada, K. *Tetrahedron* **1997**, *53*, 8149−8154, and references therein.

(3) (a) Pettit, G. R.; Kamano, Y.; Herald, C. L.; Tuinman, A. A.; Boettner, F. E.; Kizu, H.; Schmidt, J. M.; Baczynskyj, L.; Tomer, K. B.; Bontems, R. J. *J. Am. Chem. Soc.* **1987**, *109*, 6883−6885. (b) Bagniewski, P. G.; Reid, J. M.; Pitot, H. C.; Sloan, J. A.; Ames, M. M. *Proc. Am. Assoc. Cancer Res.* **1997**, *38*, 221−222.

(4) (a) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1221−1225. (b) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. *J. Nat. Prod.* **1998**, *61*, 1075−1077. (c) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 655−658. (d) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J. *J. Nat. Prod.* **1999**, *62*, 1702−1706. (e) Mitchell, S. S.; Faulkner, D. J.; Rubins, K.; Bushman, F. D. *J. Nat. Prod.* **2000**, *63*, 279−282.

(5) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L. *J. Nat. Prod.* **2000**, *63*, 611−615.

(6) Corbett, T. H.; Valeriote, F. A.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Lowichik, N.; Knight, J.; Bissery, M.-C.; Wozniak, A.; LoRusso, P.; Biernat, L.; Polin, D.; Knight, L.; Biggar, S.; Looney, D.; Demchik, L.; Jones, J.; Jones, L.; Blair, S.; Palmer, K.; Essenmacher, S.; Lisow, L.; Mattes, K. C.; Cavanaugh, P. F.; Rake, J. B.; Baker, L. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*; Valeriote, F. A., Corbett, T. H., Baker, L. H., Eds.; Kluwer Academic Publishers: Norwell, 1992; pp 35−87.

(7) Pettit, G. R.; Kantoci, D.; Herald, D. L.; Barkóczy, J.; Slack, J. A. *J. Liquid Chromatogr.* **1994**, *17*, 191−202.

(8) The NMR chemical shifts for C/H-28 and C/H-29 in ref 4b had been misassigned and should be reversed. The numbering of C/H-4 and C/H-5 for the structures in ref 4b must be switched.

(9) Additional amounts of **2** were isolated similarly as described here from Hawaiian *S. hydnoides* samples collected off the west coast of the island of Oahu.

(10) (a) Pettit, G. R.; Srirangam, J. K.; Barkoczy, J.; Williams, M. D.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Hogan, F.; Bai, R.; Chapuis, J.-C.; McAllister, S. C.; Schmidt, J. M. *Anti-Cancer Drug Des.* **1998**, *13*, 243−277. (b) Miyazaki, K.; Kobayashi, M.; Natsume, T.; Gondo, M.; Mikami, T.; Sakakibara, K.; Tsukagoshi, S. *Chem. Pharm. Bull.* **1995**, *43*, 1706−1718.

(11) Sone, H.; Shibata, T.; Fujita, T.; Ojika, M.; Yamada, K. *J. Am. Chem. Soc.* **1996**, *118*, 1874−1880.

(12) Bowman, R. E.; Stroud, H. H. *J. Chem. Soc.* **1950**, 1342−1345.

(13) For physical data of **2**, see ref 4b.

(14) Corbett, T.; Valeriote, F.; LoRusso, P.; Polin, L.; Panchapor, C.; Pugh, S.; White, K.; Knight, J.; Demchik, L.; Jones, J.; Jones, L.; Lisow, L. In *Anticancer Drug Development Guide: Preclinical Screening, Clinical Trials, and Approval*; Teicher, B., Ed.; Humana Press Inc.: Totowa, NJ, 1997; pp 75−99.

(15) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

(16) Mooberry, S. L.; Tien, G.; Hernandez, A. H.; Plubrukarn, A.; Davidson, B. S. *Cancer Res.* **1999**, *59*, 653−660.

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